# In-situ Solid-Phase Extraction and Laboratory Analysis of Ultra-trace Synthetic Musks in Municipal Effluent using GC/MS in the Full-Scan Mode

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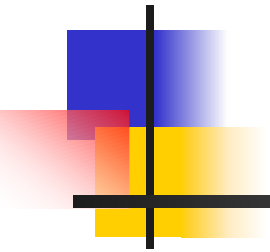

**http://www.epa.gov/nerlesd1/chemistry/pharma/index.htm**

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# Pharmaceuticals and Personal Care Products (PPCPs) as Environmental Pollutants

◆ *Pollution from Personal Actions, Activities, and Behaviors* ◆



Origins and Fate of PPCPs in the Environment

- About this Site
- Frequently Asked Questions from the Public, Media, and Scientific Communities
- NEW -- Book on Pharmaceuticals in the Environment, from the *American Chemical Society*
- Slide Presentation by Christian Daughton: **Pharmaceuticals & Personal Care Products (PPCPs) as Environmental Pollutants**

Adobe Acrobat Reader is required to view PDF documents. The most recent version of the Adobe Acrobat Reader is available as a free download. An Adobe Acrobat plug-in for assisted technologies is also available. EXIT EPA

- Critical Review Article by Daughton and Ternes: **Pharmaceuticals & Personal Care Products in the Environment: Agents of Subtle Change?**
  -- Summary/Background
- Origins and Fate of PPCPs in the Environment
- Use of PPCPs in the Environment as Analytical "Tools"

*(Continued)*



- Multidimensional Science Issues Relevant to Regulatory Considerations
- NEW - "Emerging" Pollutants, and Communicating the Science of Environmental Chemistry and Mass Spectrometry
- Scientific Conferences Devoted to PPCPs in the Environment
- Measurement Prefixes Used in Analytical Chemistry
- Laboratory/Monitoring Research in Environmental Chemistry Branch
- Listing of Scientists Involved with Environmental Aspects of PPCPs
- Opportunities for Funding and for Research in Collaboration with EPA Scientists
- Assistance with Conferences, Seminars, or Lectures
- Relevant Websites
- EPA's Terms of Environment (glossary of technical terminology)
- PPCPs and One Approach of EPA/ORD's to "Emerging" Science Issues

This web site is a dynamic "work in progress". Much of its content is continually added to and updated as more information becomes available, as more insights are acquired, and as feedback is received. With this in mind, all the information on this site (except that which has been published in the referred literature and that provided by other researchers) should be considered as "draft" and thereby subject to modification. Also note that the thrust of this web site is that of the science associated with the broad topic of PPCPs in the environment. No aspect of the materials provided by EPA employees on this site should be construed as representing thinking or positions regarding policy. This point is codified in the mission of the Office of Research and Development (ORD), which sponsors this web site. ORD is charged with developing the science that may or may not be used in the future by the Agency's regulatory Program Offices and Regions to establish, modify, or carry out national environmental policies. This web site should instead be viewed as a forum or clearinghouse for conveyance of information and opinions with the primary intention of furthering a scientific dialog on the topic of PPCPs in the environment.

*http://www.epa.gov/nerlesd1/chemistry/pharma/index.htm*

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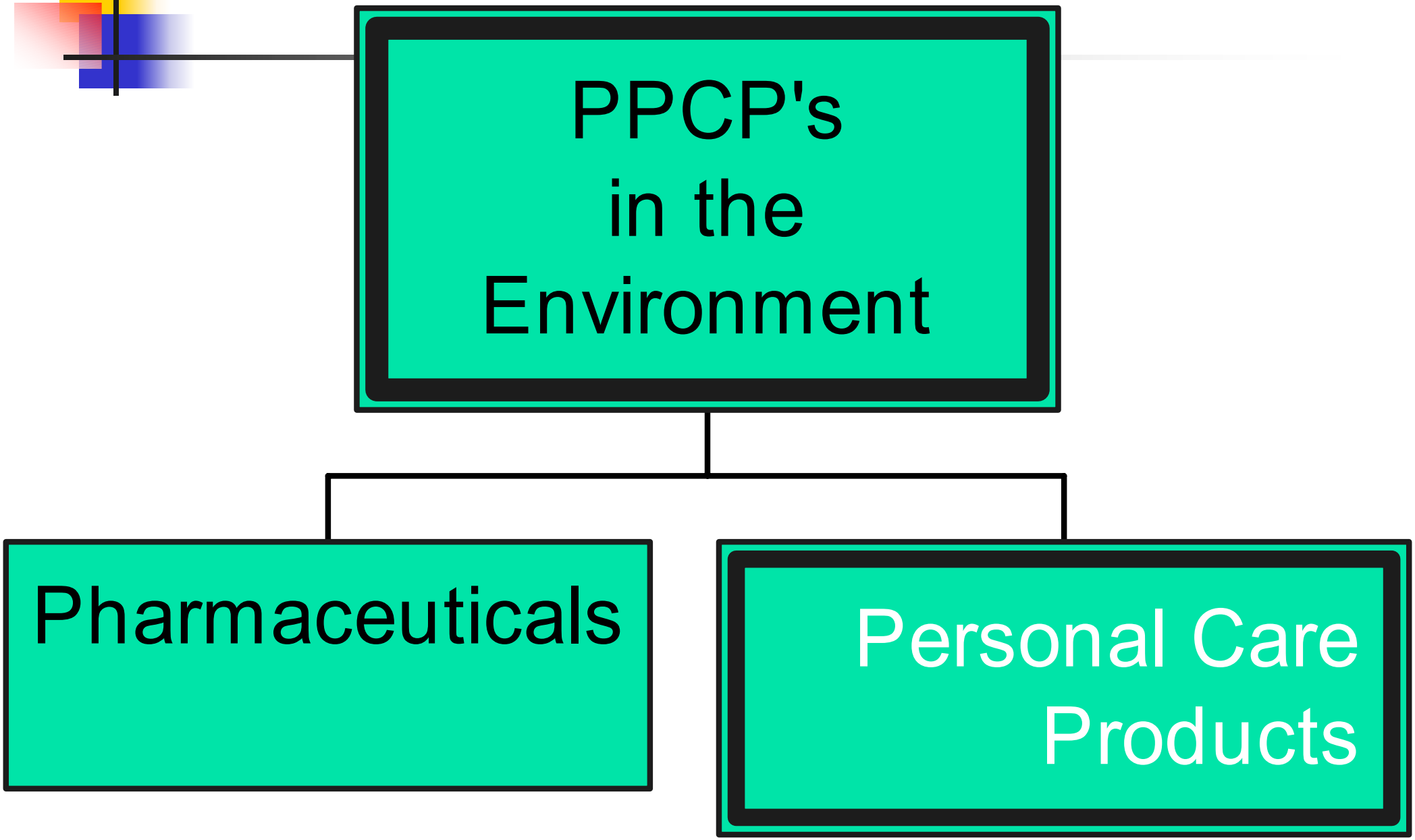
Last Updated: 09/05/01

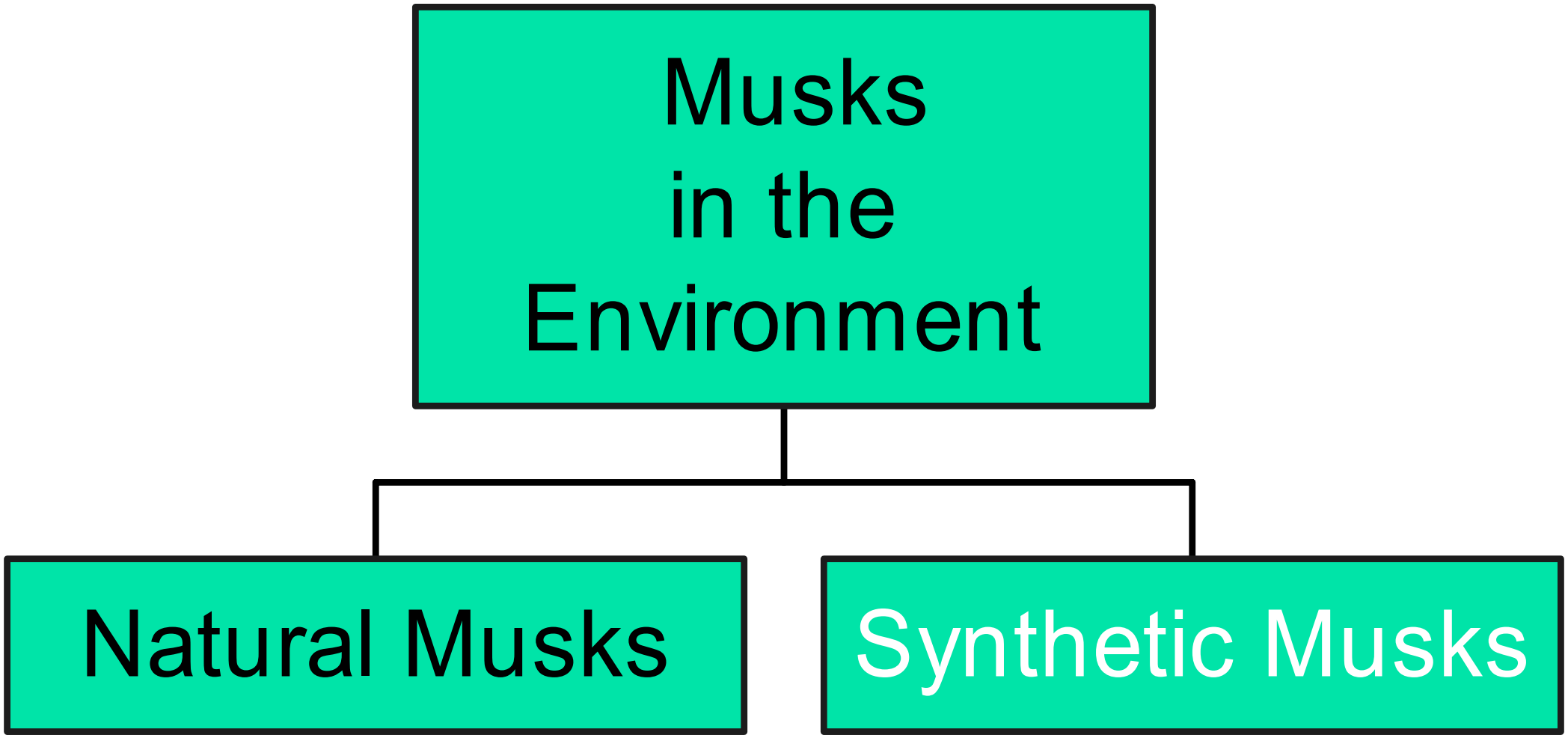
# OVERVIEW

- Musks! What are they?
- Why should we care about them?
- How do they get into the aquatic environment?
- Solid phase extraction methodology
- Summary and conclusion

# Pharmaceuticals and Personal care Products in the Environment



# Fragrance Material



# Synthetic musks

- Polycyclic and Nitro musks
- Mimic the strong and persistent aroma of the more expensive natural musks
- Synthesized by the chemical industry as inexpensive substitutes for natural musks.

# Uses of synthetic musks

- Additives for perfumes
- Detergents and bath soaps
- Body lotions and hair creams
- Fish baits and herbicides

# Where are they?

- Sewage influent/effluent. **(Gatermann et al., 1998)**
- Sewage sludge. **(Herren and Berset, 1999)**
- Rivers/Sediments. **(Rimkus et al., 1999)**
- Sediments. **(Fromme et al., 2001)**
- Fish adipose tissue. **(Yamagishi et al., 1981)**
- Human breast milk. **(Rimkus et al., 1994)**
- Human adipose tissue. **(Muller et al., 1996)**

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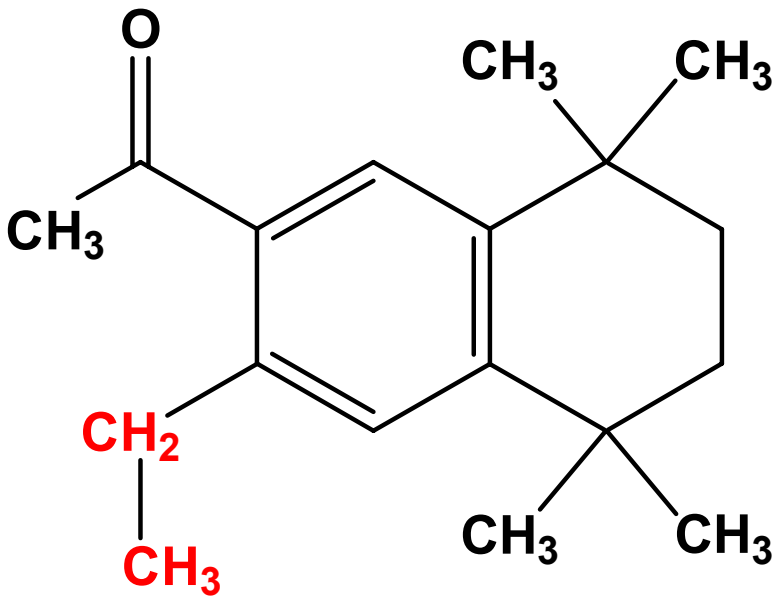
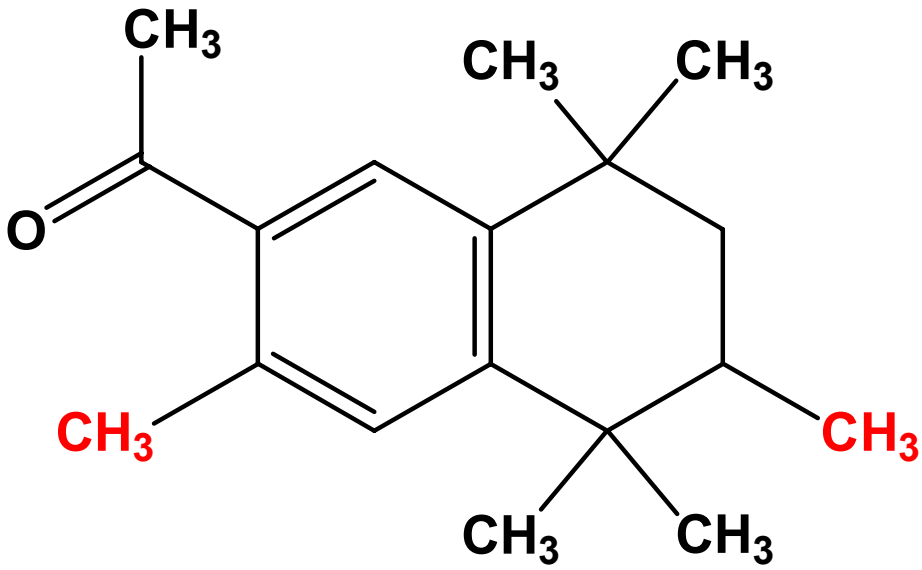
# Potential Environmental Hazards

- Bioconcentration in the adipose tissues of aquatic organisms.(Rimkus et al., 1997)
- Musk ambrette is neurotoxic. It causes the paralysis of the hind limbs in the female rats, and atrophy of the testes in the male rats.(Davis, 1967) In 1985, usage stopped by IFRA.
- In 1977, Versalide was voluntarily removed from the market by the Fragrance Industry due to its discoloration of internal organs (Opdyke, 1979), demyelination of PNS and CNS, and cumulative neurotoxicity in rats.(Spencer et al., 1979)

# Chemical Structures of Synthetic Polycyclic Musks




**Versalide®**

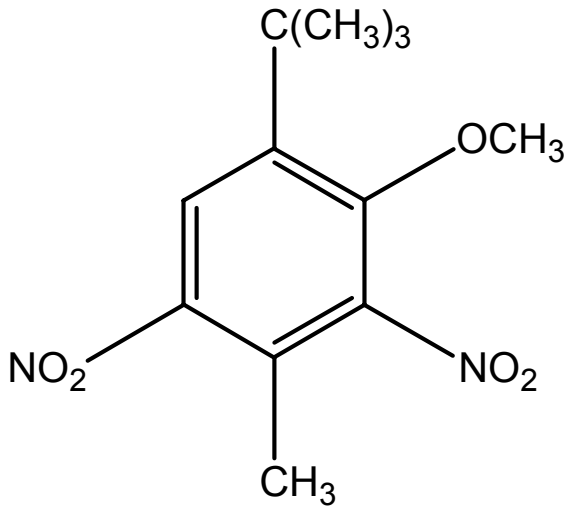



**Tonalide®**

# Chemical Structures of Synthetic Nitro Musks

**$C_{12}H_{16}N_2O_5$**
**Mol. Wt.: 268.26880**

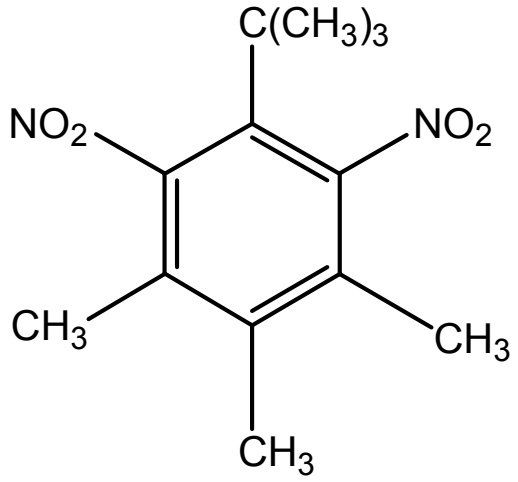
**2,6-dinitro-3-methoxy-4-tert-butyl toluene**




**Musk ambrette**

**$C_{13}H_{18}N_2O_4$**
**Mol. Wt.: 266.29620**

**1-tert-butyl-2,6-dinitro-3,4,5-trimethylbenzene**

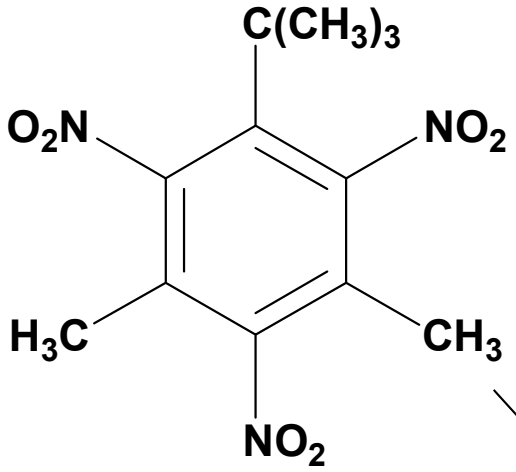



**Musk tibetene**

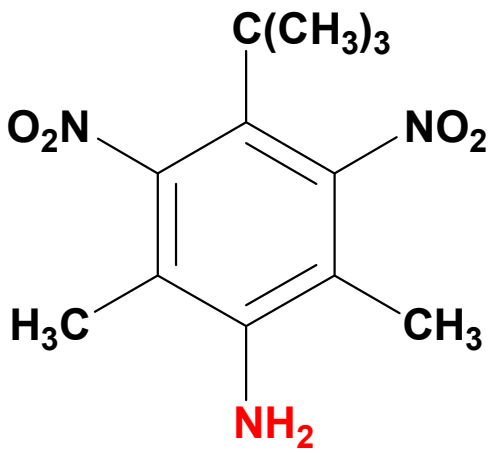
# Chemical Structures of Musk Xylene and Metabolites

**(Musk Xylene)**

**1-*tert*-Butyl-3,5-dimethyl-2,4,6-trinitro-benzene**

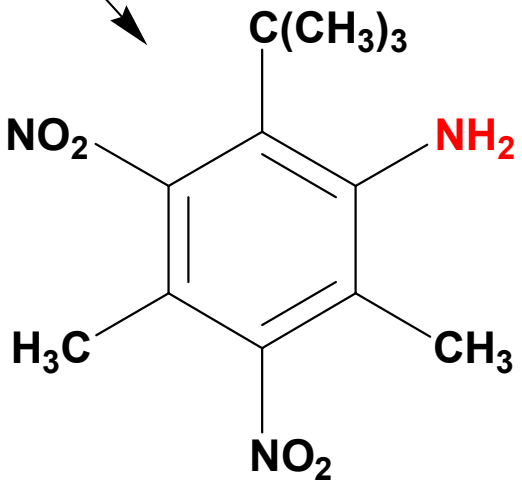



**Nitro-reductase enzymes**




**(4-AMX)**

**4-*tert*-Butyl-2,6-dimethyl-3,5-dinitro-phenylamine**




**(2-AMX)**

**2-*tert*-Butyl-4,6-dimethyl-3,5-dinitro-phenylamine**

# Qualitative and Quantification

- To perform human health risk assessment for these compounds, we need tools to identify and quantify them in their various environmental compartments.

# Previous studies

- About 30 years ago, Junk et al*., Muskty, and Nickless pioneered the use of SPE for direct extraction/enrichment of environmental samples.
- Early studies focused on Amberlite XAD-2 & 4.
- In 1979, Rees and Au**, used methanol/XAD-2 slurry for good recoveries of pesticides.
- In 2001, we evaluated several sorbents and identified poly(methylmethacrylate) mixed 1:1 with poly(styrene-divinylbenzene) as a superior sorbent for for the extraction of synthetic musks.
- Commercially available as *Varian Abselut NEXUS*

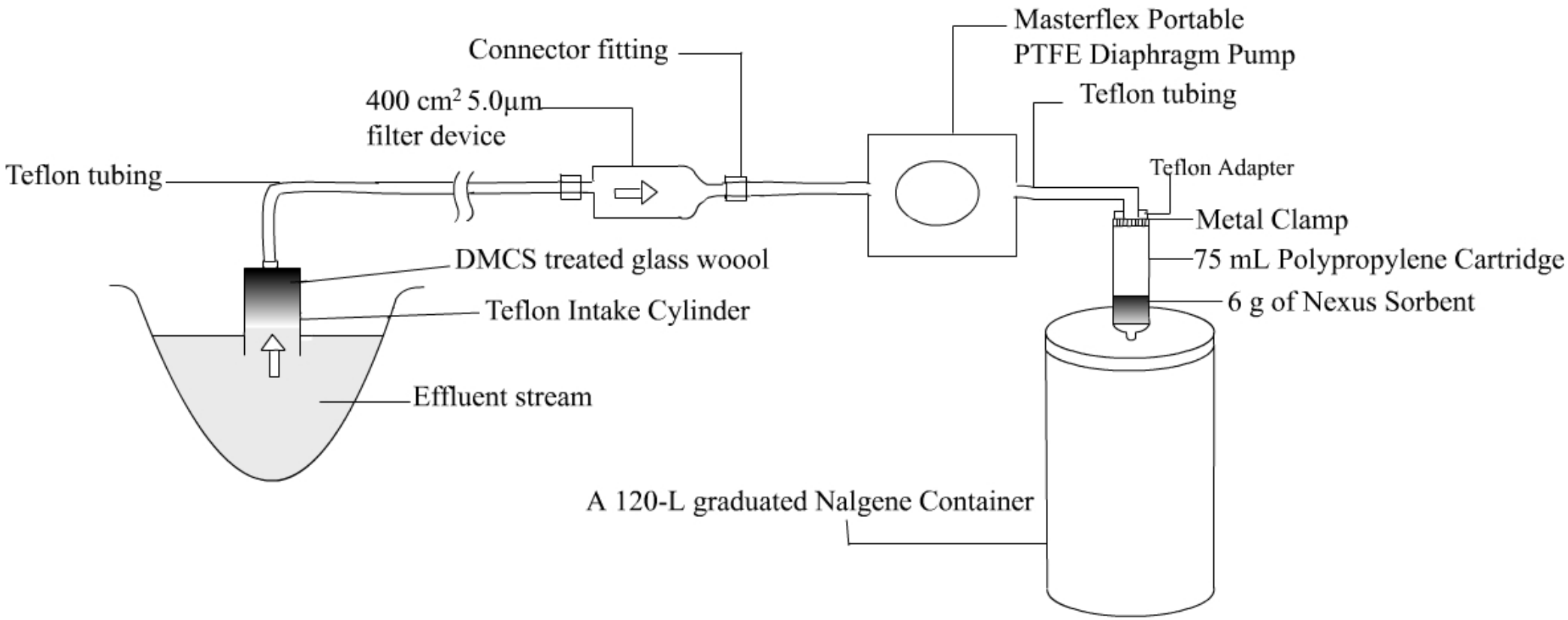
*G.A. Junk, J.J. Richard, M.D. Grieser, D. Witiak, J.L. Witiak, M.D. Arquello, R.Vick, H.J. Svec, J.S. Fritz, G.V. Calder, J. Chromatogr. 199 (1974) 745.

**V. Rees, L. Au, Bulletin of Environmental Contamination and Toxicology. 22 (1979) 561

# Recent Studies of Others

- Synthetic musks are hydrophobic (Log $K_{ow}$ 4.3 to 6.3)
- Liquid-liquid extraction of unfiltered samples yielded higher concentration in surface water
- Select (high industry volume) musks compounds were extracted with silica disk.

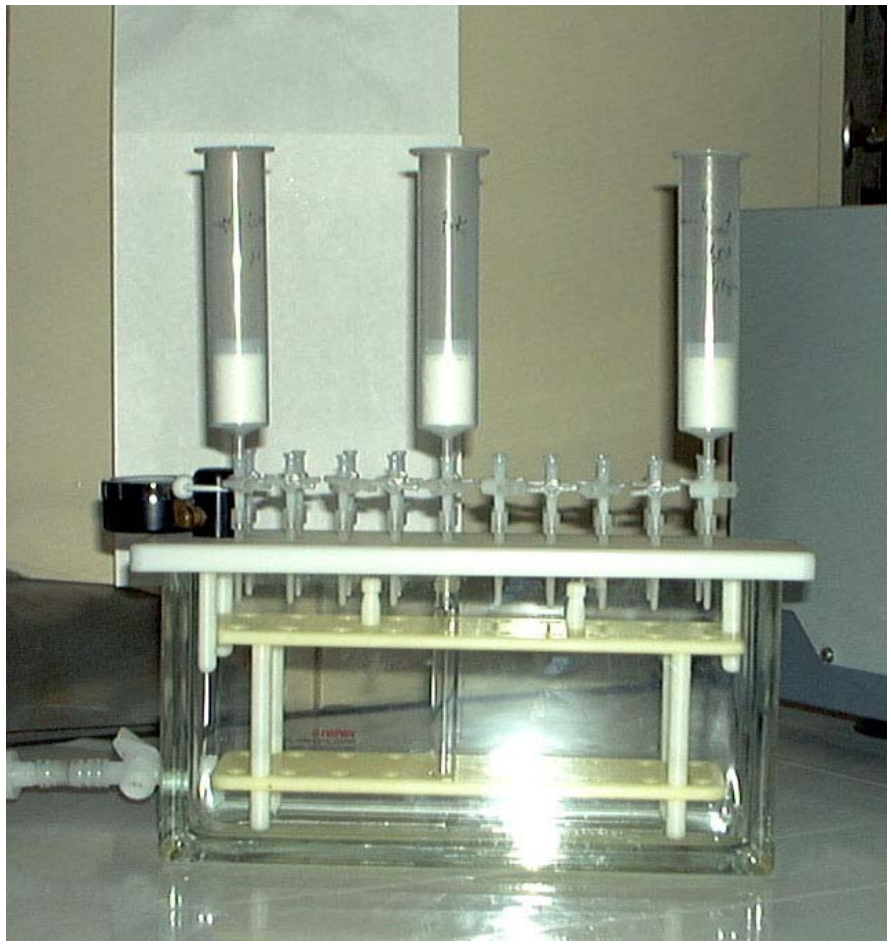
**Fig. 1** **On-Site Solid-Phase Extraction Assembly**



# In-situ Extraction of Synthetic Musk Compounds

# Elution of Analytes

- 60 mL hexane
- 60 mL ethyl acetate
- Vacuum at
  252 mm Hg

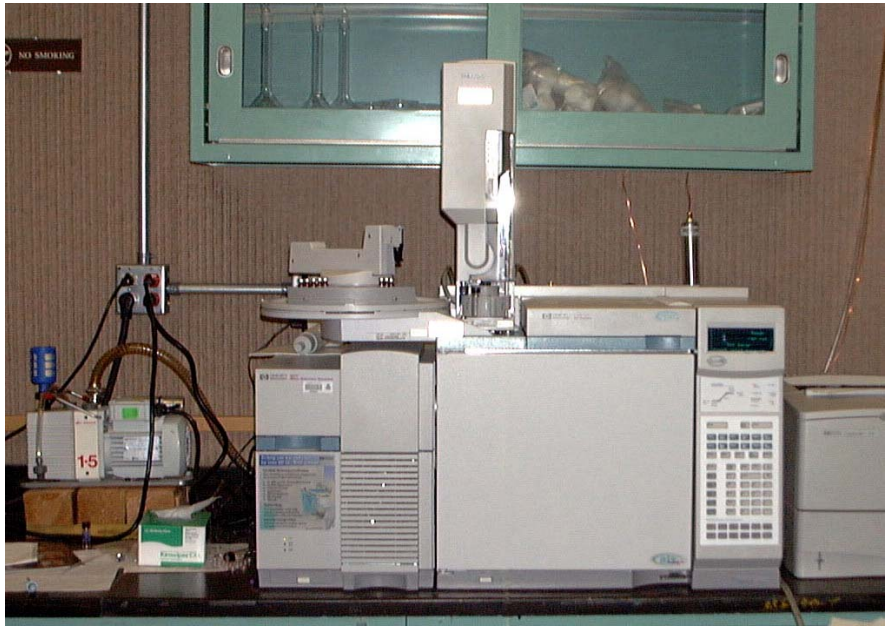


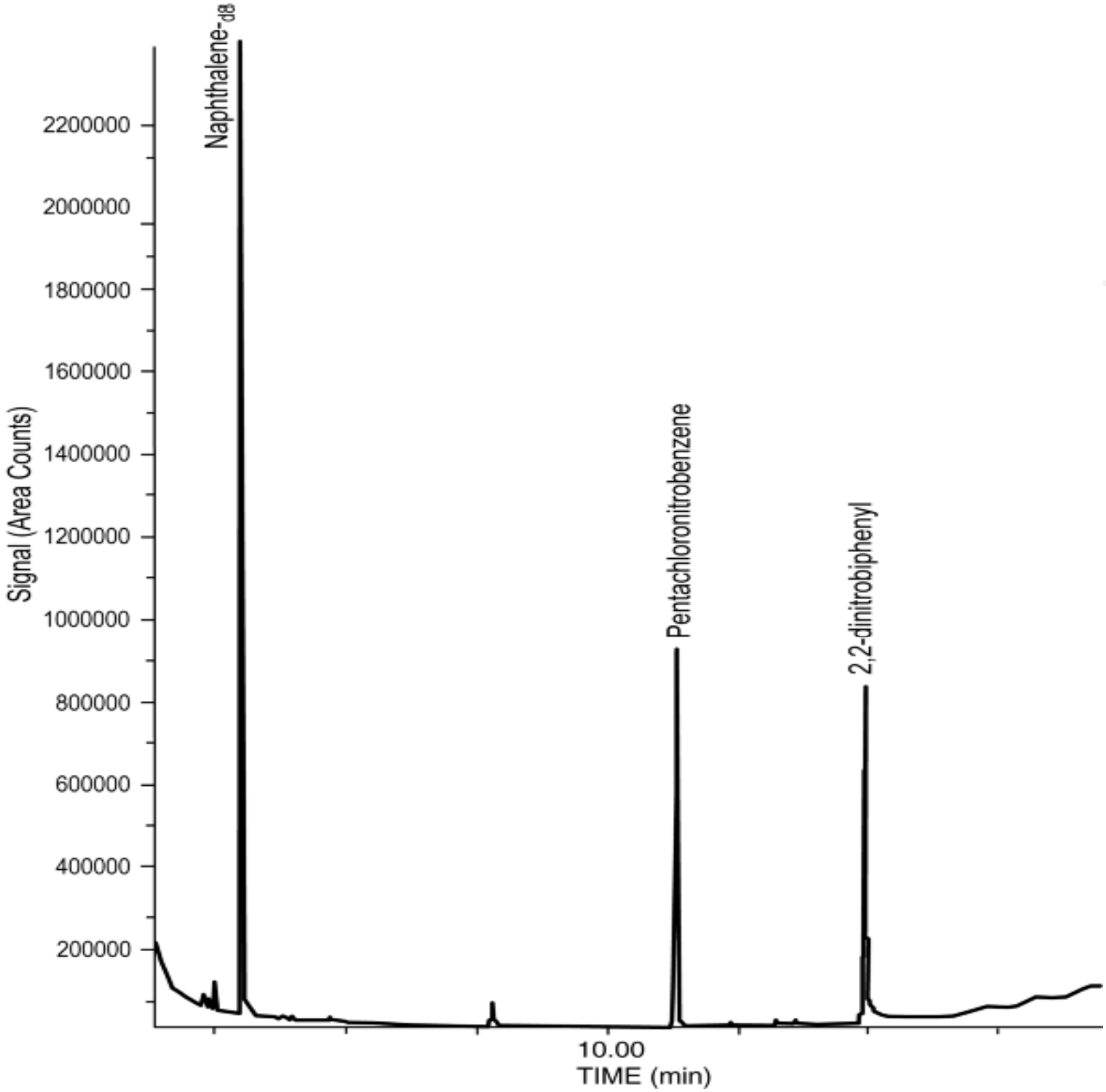
# Turbo-vap™ Solvent Exchanged

- Temperature = 30°C
- Chiller = 10°C
- Endpoint = Sensor
- Fan = 4000 rpm
- 0.4 mL, add 1 mL toluene

# GC/MS Analysis

- Injection port =250°C
- 90°C for 1min, ramp at 10°C to 300°C, hold for 5 min.
- Interface =300°C.
- 2 µL sample injected.




Naphthalene-d8
Pentachloronitrobenzene
2,2-dinitrobiphenyl
Signal (Area Counts)
2200000
2000000
1800000
1600000
1400000
1200000
1000000
800000
600000
400000
200000
10.00
TIME (min)

# Ions used.

TABLE 4. CHARACTERISTIC IONS FOR SEMIVOLATILE COMPOUNDS

| Compound | Retention Time (min) | Primary Ion | Secondary Ion (s) |
|---|---|---|---|
| Naphthalene-$d^8$ (I.S.) | 4.33 | **136.10** | 135.10, 137.10, 108.10 |
| Cashmeran | 8.01 | **191.10** | 192, 135.10, 107 |
| Celestolide | 10.46 | **229.10** | 224.10, 173, 230.1 |
| Phantolide | 10.91 | **229.10** | 224.10, 187.10, 230 |
| Pentachloronitrobenzene (Surr.) | 11.06 | **236.80** | 294.80, 213.8, 265 |
| Versalide | 11.45 | **243.20** | 244.20, 258.20, 259.20 |
| Musk ambrette | 11.69 | **253.00** | 268.00, 254.00, 251.00 |
| Traseolide | 11.84 | **215.10** | 216.10, 173.00 |
| Galaxolide | 11.89 | **243.10** | 258.10, 213.10, 244.00 |
| Tonalide | 12.00 | **243.10** | 258.10, 244.00, 201.10 |
| Musk xylene | 11.99 | **282.00** | 297.10, 283.10 |
| Musk moskene | 12.23 | **263.10** | 278.10, 264.00, 221.00 |
| MuskTibetene | 12.76 | **251.00** | 266.10, 252.00, 115.00 |
| Musk ketone | 13.17 | **279.00** | 294.10, 128.00, 280 |
| 2,2-dinitrobiphenyl (Surr.) | 13.95 | **198.00** | 168.00, 139.00, 115.00 |
| 2-Amino musk xylene | 14.51 | **264.10** | 249.10, 215.10, 191.00 |
| Amino musk ketone | 14.36 | **267.10** | 252.10, 218.10, 160.10 |
| 4-Amino musk xylene | 14.92 | **252.00** | 267.10, 218.00, 235.00 |

I.S. = internal standard

Surr. = Surrogate

**Table 5. % Spike Recovery data from 60 L Sample* (n=3)**

| Analytes | Nanopure water (%RSD) | STP Effluent (%RSD) | MDL (ng/L) |
|---|---|---|---|
| Musk xylene | 102 (4) | 97 (6) | 0.02 |
| Musk ketone | 98 (3) | 95 (5) | 0.20 |
| Musk ambrette | 101 (5) | 96 (7) | 0.30 |
| Musk moskene | 96 (4) | 92 (4) | 0.03 |
| Musk tibetene | 98 (2) | 95 (7) | 0.02 |
| Versalide® (AETT) | 99 (5) | 96 (6) | 0.02 |
| Galaxolide® (HHCB) | 99 (3) | 97 (5) | 0.02 |
| Phantolide® (AHDI) | 97 (3) | 91 (5) | 0.02 |
| Cashmeran® (DPMI) | 99 (4) | 94 (6) | 0.02 |
| Celestolide® (ADBI) | 98 (3) | 96 (8) | 0.02 |
| Traseolide® (ATII) | 95 (5) | 90 (8) | 0.02 |
| Tonalide® (AHTN) | 107 (3) | 94 (6) | 0.02 |
| 4-Amino musk xylene | 87 (5) | 80 (10) | 0.30 |
| 2-Amino musk xylene | 89 (7) | 82 (11) | 0.25 |
| Amino musk ketone | 90 (6) | 92 (8) | 0.25 |

* Recovery data from extraction through GPC steps.

## Table 6. Concentrations (ng/L) of synthetic musk compounds, and nitro musk metabolites in STP effluent stream.

| Analytes | 85 L[a] | 65 L[a] | 85 L[b] | 45 L[b] | 60 L[c] (%RSD) |
|---|---|---|---|---|---|
| Musk xylene | **1.3** | < MDL | < MDL | **0.5** | < MDL |
| Musk ketone | **27.5** | **21.5** | **23.4** | **21.3** | < MDL |
| Musk ambrette | < MDL | < MDL | < MDL | < MDL | < MDL |
| Musk moskene | < MDL | < MDL | < MDL | < MDL | < MDL |
| Musk tibetene | < MDL | < MDL | < MDL | < MDL | < MDL |
| Versalide | < MDL | < MDL | < MDL | < MDL | < MDL |
| Galaxolide | **138** | **111** | **152** | **35.0** | **40.8 (1.8)** |
| Phantolide | **4.3** | **3.1** | **5.0** | **2.5** | **2.4 (4.3)** |
| Cashmeran | < MDL | < MDL | < MDL | < MDL | < MDL |
| Celestolide | **2.1** | **0.3** | **0.3** | **0.5** | **1.4 (7.2)** |
| Traseolide | **83.8** | **34.5** | **126** | **6.6** | < MDL |
| Tonalide | **67.3** | **47.1** | **92.2** | **26.6** | **36.8 (2.5)** |
| 4-Amino musk xylene | **1.4** | **11.6** | < MDL | **31.5** | < MDL |
| 2-Amino musk xylene | < MDL | < MDL | **0.9** | < MDL | < MDL |
| Amino musk ketone | < MDL | < MDL | < MDL | < MDL | < MDL |

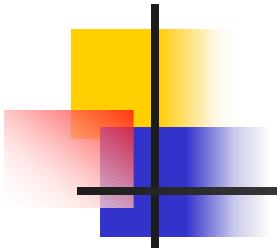
[a] Effluent sample downstream from a tertiary sewage treatment plant's discharge pipe. (n=1)

[b] Effluent sample taken 14 days later from same location. (n=1)

[c] Effluent sample near a different tertiary sewage treatment plant's discharge pipe. (n=3)

# Summary

- Enrichment factor approaches $10^6$ : 1, without methanolic pretreatment or activation of resin
- This simplified process leads to the extraction of dissolved-phase analytes only (particulates > 5.0 µm excluded)
- Resultant sample is highly compact, easily stored, and transported
- High signal/noise enables unknown to be authenticated with NIST mass spectral library
- Structures are easily proven by showing identity to authentic standards (GC- ret. time and full mass spectrum)



Osemwengie, L.I., and S. Steinberg. On-Site Solid Phase Extraction and Analysis of Ultra-trace Synthetic Musks in Municipal Sewage Effluent Using Gas Chromatography-Mass Spectrometry, Full-scan Mode. "Journal of Chromatography A", Vol 932/1-2, pp 107-118 (2001).

# Acknowledgement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